# Investigation of drug partition kinetics to fat in simulated fed state gastric conditions based on drug properties.

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**Abstract:** The presence of fat in the gastric environment can affect the pharmacokinetic behavior of drugs with mechanisms which have not been yet fully understood. The objective of the current study was to assess the drug partition to the lipid part of the fed gastric content under different emulsification conditions, using *in vitro* discriminating setups. The model drugs used in the study were selected on the basis of different physicochemical properties (lipophilicity, ionization, molecular weight and aqueous solubility) and different food effect observed in *in vivo* human studies. Fed State Simulated Gastric Fluid prepared with skimmed milk ($FeSSGF_{sk}$) and anhydrous milk fat were used as surrogates for the aqueous and fat portions of the fed gastric environment respectively. An optimized biphasic model was developed so as to predict the differences in partition rate constants to fat, for model drugs of a wide range of the properties mentioned above. The experimental data and the use of statistical analysis revealed that molecular weight, molecular weight and log $D_{pH\ 5}$ interaction and negative food effect act as negative factors to the rate constants of fat partition, while absence of food effect and $logD_{pH\ 5}$ interaction with aqueous solubility affect the rate constants of partition to fat favorably.

**Keywords:** Fed state, Physicochemical properties, Food effect, Drug partition, Partial least squares regression

**Abbreviations**

**aq sol $_{pH\ 5}$:**calculated drug aqueous solubility at pH 5 (μg/mL), **$C_t$**:drug percentage partitioned to fat at time t, **$C_{max}$**:maximum drug percentage partitioned to fat, **DModY**:distance to model; residuals of Y, **$FeSSGF_{sk}$**:Fed state simulated gastric fluid prepared with skim milk, **GF/D**:glass microfiber, **HGL**:human gastric lipase, **$k_{part}$:**first-order partition rate constant, **log D $_{pH\ 5}$**:octanol/water distribution coefficient at pH 5, **log P**:octanol/water partition coefficient, **MW**:molecular weight, **MWCO:**molecular weight cut off, **PLS**:partial least squares, **PRESS:**predicted residual error sum of squares, **$Q^2$**:crossvalidated coefficient of determination, , **$R^2$**:coefficient of determination, **RC**:regenerated cellulose, **$R_e$**:Raynold's number, **RN**:Rhizopus niveus, **rpm**:rotations per min, **SLS**:sodium lauryl sulfate

# 1. Introduction

The oral route is considered the most common route of drug administration, due to its convenience, lower cost of formulations developed and patient compliance. Drug solubility, dissolution and permeability are critical processes taking place in the gastrointestinal tract and determine the drug's bioavailability. For most solid dosage forms (with the exception of orodispersing formulations), the stomach is the part of the GI tract where dissolution begins as the volumes and transit time in the oral cavity and oesophagus are insignificant. The stomach can be divided in three distinct parts: fundus, corpus and antrum; the fundus acts as a gastric reservoir, the antrum is the site where trituration and particle size reduction takes place, while the corpus connects these two parts (Koziolek et al., 2013). Food forms layers in the stomach, with fat floating on top of an aqueous layer and heavier particles sedimenting in the sinus (Schulze, 2006), while the aqueous layer contains small particles which are emptied from the stomach as the gastric emptying process takes place (Koziolek et al., 2013). The lipid part of the meal administered has a prominent role in a potential drug food effect through many possible mechanisms. Some common ones involve the increase of lymphatic transport of drugs (Hunt and Knox, 1968), the constriction of intestinal efflux transporters and the formation of intestinal mixed micelles (bile salt/phospholipids/cholesterol) as a result of exogenously administered lipids (Porter and Charman, 2001). Presence of fat in the stomach increases the gastric residence time of drugs thus allowing more time for drug dissolution. Moreover, the presence of lipid components in meals can modify the *in vivo* behaviour of certain molecules either promoting the formation of mixed micelles with bile salts or by drug solubilisation by fat.

Thus, when developing *in vitro* predictive tests towards the evaluation of drug dissolution in the GI tract it is important to simulate the effect of the lipid part of the meal, incorporating it to the dissolution media used, with an aim to predict effectively possible food

effects on their pharmacokinetic behaviour. Since fat can improve the dissolution characteristics of poorly soluble drugs in the stomach, the knowledge of possible drug-lipid interactions is essential. Also, knowing the rate that the drug partitions to fat is equally important, as the meal remains in the gastric compartment for 1-4 hours (depending on the type of meal) (Read et al., 1986), with one part of the released drug being dispersed in the lipid phase and the rest solubilised or precipitated in the aqueous gastric phase. Of the total solubilised amount of drug, which includes both the free and partitioned drug, only the free fraction has the potential to be absorbed in the intestine (Porter et al., 2007). It is therefore important to determine the parameters which control drug-lipid interactions and evaluate possible dependence of drug physicochemical properties. Knowledge of the physicochemical parameters controlling this type of interactions can shed light towards the understanding of the mechanisms inducing positive or negative food effect after drug co-administration with high-fat meals.

Another factor which can affect drug dissolution and permeability in the gastrointestinal tract is the presence of lipolytic enzymes. Lipid digestion starts in the oral cavity with the help of lingual lipases and continues in the stomach from lingual and gastric lipases; while the major part of the lipolysis process takes part in the small intestine, where drug absorption takes place, primarily by the pancreatic lipase (Iqbal and Hussain, 2009). Gastric lipolysis was believed to account for 10-20% of the total lipolysis process in the GI tract (Carriere F. et al., 1993; Schonheyder and Volqvartz, 1946). More recent evidence though, showed that human gastric lipase (HGL) may be responsible for up to 40 % of the total lipolytic activity (Armand, 2007). Gastric lipase hydrolyses long and medium-chain to diglycerides, monoglycerides and fatty acids. These digestion products along with the shear forces developed in the stomach during digestion lead to fat emulsification, creating a coarse lipid emulsion (Thomas et al., 2012). Because of the limited role of HGL in the dissolution of conventional tablets, its use in gastric

dissolution media has been relatively limited. Its presence though may be important for lipid-based drug delivery systems (Pedersen et al., 2013) and possibly in the prediction of drug dissolution behaviour after administration of lipid rich meals, as these ezymatic processes take place in both cases (lipids derived from food or lipid based formulations) (Rezhdo et al., 2016). In the fed state, gastric lipase contributes to a greater extent to the total lipolysis, due to higher HGL stimulation at higher pH values, with its activity measured more than 10 times higher at pH=5.4 than at pH=2.8 (Pedersen et al., 2013).

In the fed stomach, un-digested fat forms a lipid layer floating on the top of the gastric content while emulsified fat particles move with the aqueous phase to the pyloric antrum; the aqueous content fills the distant antrum and moves towards the duodenum faster than fat and solid residues (Schulze, 2006). Because of the complex stratification of aqueous phase and un-digested and emulsified fat in the fed stomach, it is important to assess the interactions formed between the drugs and each of the phases so as to explain certain lipid-induced changes in drugs' pharmacokinetic parameters and also problems in the analysis of biorelevant media related to the presence of fat.

The purpose of the current work was to study the role of the lipid part of a meal, simulated in a gastric fed state medium, in inducing changes of drug pharmacokinetic parameters. The percentage of drug partitioned to fat from the "aqueous" phase of the stomach under physiological conditions in the fed state was determined using three different experimental setups and 15 model compounds with a wide range of physicochemical properties were used. The effects of fat emulsification on drug partitioning to fat was also evaluated by performing experiments in the presence of surfactants. Statistical tools [partial least squares (PLS) regression analysis] were used to assess the impact of related drug physicochemical properties and drug food effect observed *in vivo* (changes in the drug's pharmacokinetic behaviour after meal administration) on drug partition in fat.

## 2. Materials and Methods

### 2.1. Materials

Atorvastatin calcium salt trihydrate (≥ 98% (HPLC)), danazol (≥ 98%), furosemide (≥ 98%), phenytoin (pharmaceutical secondary standard; traceable to USP and PhEur), itraconazole (≥ 98% (TLC)), propafenone hydrochloride (≥ 98% (HPLC)), indomethacin (≥ 99%) and indoprofen (analytical standard) were purchased from Sigma- Aldrich, UK, while nifedipine (98.0-102.0% (on dried substance)) and ketoconazole (inclusive between 98.0%) from Fisher Scientific, UK. Griseofulvin (> 97%) and felodipine were purchased from Alfa Aesar, UK and ibuprofen (97-103%) was purchased from Fagron, UK. MK-C1 and MK-C4 were provided by Merck & Co, US.

Sodium acetate trihydrate, sodium chloride, hydrochloric acid (36.5-38%), glacial acetic acid (≥ 99%), sodium dodecyl sulphate (S/5200/53) and all phosphate salts were purchased from Fisher Scientific, UK. HPLC grade methanol, acetonitrile, trifluoroacetic acid (≥ 99.0%), diethylamine (≥ 99.5%), thriethylamine (≥ 99.5%) and formic acid were all purchased from Sigma- Aldrich, UK.

Cronus 13 mm regenerated cellulose (RC) syringe filters 0.45 µm were purchased from LabHut Ltd, UK and 2.7 µm GF/D (glass fiber) filters from Fisher Scientific, UK.

Lipase from *Rhizopus niveus* (Lipase RN, approximately 83 kDa, cat# 62310) and calcium chloride dehydrate (≥ 99.0%) were purchased from Sigma–Aldrich, UK. Sainsbury's < 0.1% fat UHT milk and Sainsbury's anhydrous milk fat were commercially purchased (Sainsbury's, UK). Dialysis tubing cellulose membrane (MWCO 12000-14000, avg. flat width 25 mm (1.0 in.)) was purchased from Sigma- Aldrich, UK.

## 2.2. Instrumentation

Partition experiments were run in triplicate at 37 °C, using 50 mL Corning® PP self-standing centrifuge tubes, the USP 2 paddle apparatus (Agilent 708-DS Dissolution Apparatus) or 100 mL glass DURAN™ bottles (Fischer). All samples were analysed in an HPLC system consisting of an Agilent 1200 series binary pump (G1312A), an Agilent 1200 series DAD detector (G1315D), an Agilent 1200 series autosampler (G1329A), an Agilent 1200 series controller (G1316A) and Chemstation software (Agilent Technologies, Santa Clara, United States). A pH meter Mettler-Toledo AG (model SevenCompact pH/Ion S220, Schwerzenbach, Switzerland), a centrifuge Hereus Biofuge Primo R (Thermo Scientific, Hanau, Germany) and a vortex mixer Rotamixer (HTZ, Cheshire, UK) were used.

## 2.3. Model drugs selection

Assessment of the drug partition to fat was conducted for 15 drugs of a wide range of lipophilicity, ionisation, aqueous solubility, *and in vivo* food effect (Table 1). For drugs that were ionized in pH 5, the log $D_{pH\,5}$ was calculated based on the partition coefficient and the ionization constant of the studied drugs according to Equations 1 (weak acids) and 2 (weak bases) (Chiang and Hu, 2009):

$$\log D_{pH\,5} = \log P - \log(1 + 10^{pH-pKa}) \quad \text{for weak acids} \quad \text{(Eq 1)}$$

$$\log D_{pH\,5} = \log P - \log(1 + 10^{pKa-pH}) \quad \text{for weak bases} \quad \text{(Eq 2)}$$

For neutral drugs or for weak acids/weak bases that were unionized in pH 5, log $D_{pH\,5}$ and log P were considered equal. Working drug concentrations were selected according to experimental drug aqueous solubility values as reported in the literature or calculated values where experimental values were not available (Advanced Chemistry Development

(ACD/Labs) Software v.11.02, Sci-finder), so as to avoid possible drug precipitation as the drug was initially dissolved in the "aqueous" (the part which does not contain fat, consisting of a mixture of skimmed milk and acetate buffer, $FeSSGF_{sk}$) of the fed-state medium used in the study. Due to its extremely low solubility in water (< 0.1 μg/ mL), working concentration of MK-C4 was selected according to its solubility in the "aqueous" phase of the medium in a 24 h period, performed using the shake-flask method (Wagner et al., 2012). In summary, an excess of drug was added to the solubility medium and left to equilibrate for 24 h at 37 °C under constant shaking. An aliquot of the saturated medium was initially filtered through a GF/D 2.7 μm filter and quantified after addition of ACN (2 parts in 1 part of medium), vortex (30 sec at full speed), centrifugation (15 min, 8000 rpm, 37 °C) and finally filtration through a regenerated cellulose filter. Drug was quantified in HPLC.

### 2.4. Drug partition to fat studies

A modified version of Fed State Simulated Gastric Fluid ($FeSSGF_{sk}$; medium simulating the fed gastric composition early after drug administration (Dressman et al., 1990; Fotaki and Vertzoni, 2010) was selected in order to simulate the "aqueous" phase of the working fed state medium. $FeSSGF_{sk}$ was prepared according to Jantratid et al. (Jantratid et al., 2008) by mixing skimmed milk and acetate buffer pH = 5 at a 1:1 volume ratio. For the preparation of 1 L of medium, 500 mL milk and 480 mL acetate buffer were mixed under constant stirring using a magnetic stirrer. pH was adjusted to 5 with 1 N HCl and the volume was adjusted to 1 L with acetate buffer. Anhydrous milk fat was selected as a surrogate of the lipid phase of FeSSGF. It is a cream or butter derivative, having water and proteins removed, and contains at least 99.8% milk fat (Rønholt et al., 2013).

Three different setups were developed for the assessment of drug partition rate from the aqueous to the lipid part of the fed gastric medium (Table 2). The three setups used 50 mL

centrifuge tubes for diffusion through a dialysis membrane (setup I), the USP dissolution apparatus 2 (setup II) or 100 mL glass bottles for partition assessment of drugs in a smaller scale (setup III). For every drug, working solutions were prepared in $FeSSGF_{sk}$ and left under constant stirring at 37 °C for 60 min at concentrations equal to the drugs' aqueous solubility values. Appropriate quantities of anhydrous milk fat, equivalent to the desired w/v percentage of the total volume of each setup (pre-heated at 37 °C) (Table 2) were weighed. Appropriate volume of the $FeSSGF_{sk}$ drug solution was placed in the receptor vessel of each setup. A working temperature of 37 °C was maintained by the means of a 37 °C incubator room, a thermostated jacket or a heating plate for setups I, II and III respectively. Drug partition rate to fat was described by measuring the % decrease in the donor concentration with time, with samples taken from the middle of the $FeSSGF_{sk}$ layer at defined time points for a period of 24 or 48 hours. Experiments were performed in triplicate and % drug partition to fat was expressed as mean ± standard deviation. The exact quantities and volumes of lipid and aqueous parts, sampling time points and agitation conditions are stated in Table 2. The experimental setup suitability was evaluated with a pilot study of five drugs of different lipophilicity [propafenone hydrochloride, ketoconazole, nifedipine, danazol and atorvastatin calcium, ($\log D_{pH\ 5}$ = 0 - 4.20)].

The effect of fat percentage used was assessed in setup I (dialysis membrane setup), using nifedipine as the model drug. The quantity of fat placed in the membrane was equivalent to 5%, 8%, 15%, 20%, 25% w/v fat concentrations. 0.5 mL samples were collected from the donor compartment (30 mL of milk-based medium) at defined time-points (Table 2) and agitation was maintained by a 15 x 6 mm magnetic bar rotating at 300 rpm in a centrifuge tube with conical base and skirted bottom. Partition experiments using setup I were performed for the other four drugs of the pilot study using the 25% w/v fat concentration. The pore size of the membrane (MWCO 14000 Da), was multiple times higher than the molecular weight of the

model drug of the study (nifedipine-346.33 g/mol), and therefore allowed the process to be controlled by the affinity of drug for the receptor rather than the membrane.

The effect of agitation speed was evaluated in setup II, using the dissolution apparatus paddle at 3 different speeds (150, 200 and 250 rpm) and danazol as the model drug and fat quantity equivalent to 25% w/v concentration of the total medium (Table 2). The volume of $FeSSGF_{sk}$ used was 500 mL. As emulsification between the two phases was observed during the experiments, agitation was stopped for 2 min for the two layers to separate before sampling. 5 mL samples were withdrawn at defined time-points for a duration of 8 hours and volume was replaced with drug solution in $FeSSGF_{sk}$. Partition experiments using setup II were performed for the other four drugs of the pilot study using 150 rpm agitation speed. Setup III, like setup II, was a biphasic setup without the presence of dialysis membrane but performed at a smaller scale (30 mL of $FeSSGF_{sk}$). Agitation was provided by a 15 x 6 mm magnetic bar at 300 rpm. Agitation was paused for 2 min to allow for phase separation and 0.5 mL samples were collected from the drug donor compartment at defined time-points for a period of 8 hours.

Once optimum parameters (fat percentage, medium volume, agitation means and speed) were selected, they were applied to all 15 drugs of the study. The experimental setup and the fat percentage added for the final setup developed were selected on the basis of providing adequate discrimination among drug partition profiles and reasonable times for complete profiles.

Partition experiments in the presence of a surfactant were conducted using the partition setup III in order to assess the effect of emulsification conditions in the fed gastric environment on drug rate of partition. The partition rate constants were evaluated in the presence of an anionic surfactant (SLS) and also in the presence of a gastric lipase equivalent (RN lipase) dissolved in a $CaCl_2$ solution added at a concentration yielding activity similar to the

physiological values (approximately 40 U/mL) (Armand, 2007; Diakidou et al., 2009a). 6 mL of a stock solution of SLS or RN lipase in acetate buffer pH 5 were added to 24 mL of drug solution in $FeSSGF_{sk}$ (same drug concentrations as in partition "control" experiments without the presence of SLS or enzyme). $CaCl_2$ was added to a total 1.4 mM concentration. The concentration of the surfactant/enzyme stock solutions were selected so as to achieve a 1% w/v (for SLS) or 40 mg/mL (for lipase) concentration in the total volume of the system. $FeSSGF_{sk}$ and surfactant/lipase were left to mix for 5 min and the fat layer (10.3 g, Table 2) was added on top of the drug donor ($FeSSGF_{sk}$ and surfactant). Agitation was stopped for 2 min and after phase separation, 0.5 mL samples were taken from the drug donor at defined time-points for a period of 8 hours. Experiments were performed in triplicate and % drug partitioned was expressed as mean ± standard deviation.

## 2.5. Sample treatment and drug quantification

Drug quantification in $FeSSGF_{sk}$: 2 parts of methanol were added to 1 part of $FeSSGF_{sk}$ immediately after its sampling and the mixture was vortexed (30 sec), centrifuged (8000 rpm, 15 min, 4 °C), filtered through a 0.45 μm RC filter and analysed with HPLC. Drug was quantified against a set of calibration standards in $FeSSGF_{sk}$ treated as described above. Modifications of published chromatographic methods were used for drug quantification (Table 3).

## 2.6. Data analysis

### *2.6.1. Data fitting*

In order to determine the rate constants of drug partition, partition data (drug % partitioned vs time) were fitted to a first-order model (Eq. 1) using GraphPad Prism® v.7 software (GraphPad, US). Goodness of fits was assessed on the basis of coefficient of determination and normality test.

$$F_t = F_{max} * (1 - e^{-k_{part}t}) \quad \text{(Eq. 3)}$$

$F_t$ is the % drug partitioned to fat at time t, $F_{max}$ is the % maximum drug partitioned to fat (Fmax was not estimated by the model but the measured maximum % of drug partitioned into the fat was used) and $k_{part}$ is the first-order partition rate constant.

Drug partition rate constants were compared using a two-way analysis of variance (ANOVA) repeated measures (multiple measures of the same variable for all the studied levels under different experimental conditions), with Bonferroni's post-hoc test, using GraphPad Prism® v.7 software. The selected factors were: i. compound (Table 1) and ii. experimental setups (setup I, II, III). The effect of surfactant/lipase was evaluated using a two-way ANOVA for the comparison of partition rate constants with Bonferroni's post-hoc test. The selected factors were: i. compound (Table 1) and ii. emulsification (surfactant, lipase). Statistical significance level was set at $p < 0.05$.

### *2.6.2. Multivariate data analysis [Partial Least Squares (PLS) regression]*

The drug partition rate constants to fat were correlated to drug physicochemical properties [lipophilicity (log $D_{pH\,5}$), aqueous solubility at pH 5 (aq $sol_{pH\,5}$), molecular weight (MW)] and food effect [negative food effect (-), absence of food effect (0), positive food effect (+)] observed in *in vivo* human studies by partial least squares (PLS) regression using the XLSTAT software (Microsoft, US). The first order partition rate constant ($k_{part}$) was set as the response. As the impact of log $D_{pH\,5}$ on drug partition to fat may differ according to the drug physicochemical properties or the *in vivo* food effect, the combined effect of two variables on the response was assessed by including interaction terms of $logD_{pH\,5}$ with aqueous solubility at pH 5 (μg/mL), molecular weight (MW) and food effect in the model. The main advantage of PLS as a regression technique is the possibility to analyse data with independent variables which may be highly collinear (Wold et al., 2001). The parameters were selected on the basis

289 of the physicochemical aspects which control drug diffusion process between an aqueous and
290 lipid layer. Assuming that the mechanism controlling the partition process is governed by the
291 same basic principles as in lipid bylayers of biological membranes, drug partition is also
292 dependent on drug diffusion coefficient and drug partition coefficient into the membrane
293 barrier. Diffusion coefficient is dependent on size, shape and solvent-drug interaction.
294 Therefore, even though log D is a good predictor for drug's partition rate to fat, other
295 parameters such as drug size and molecular weigh, which correlate to the diffusion coefficient,
296 have to be considered. *In vivo* food effect is a complex phenomenon depending on several drug
297 and/or gastrointestinal parameters and it was included in the model as an independent variable
298 to investigate the role of drug partition to fat for the observed *in vivo* food effects. Interactions
299 of log $D_{pH\,5}$ with the rest of the properties were included in the model with an aim to elucidate
300 possible drug partition mechanisms for which drug lipophilicity alone cannot account for.

301 The model quality was evaluated on the square of the coefficient of determination ($R^2$)
302 and goodness of prediction ($Q^2$). $R^2$ and $Q^2$ values close to 1 refer to a model of good fit and
303 prediction power respectively while a difference of $R^2$ and $Q^2$ lower than 0.2-0.3 between them
304 is indicative of a successful model (Eriksson and Umetrics, 2008). A $Q^2$ value > 0.5 was
305 considered acceptable for good model predictability (Roy et al., 2015). Full cross-validation
306 (leave-one-out procedure) was used to develop and evaluate the regression model. The
307 optimum number of calibration factors (principal components) for each model was selected
308 based on the model's optimum predictability ($Q^2$) and predicted residual error sum of squares
309 (PRESS). Lower PRESS values indicate better prediction (Krishnan et al., 2011), with the
310 number of latent variables where PRESS starts increasing indicating the number of variables
311 which to be retained in the model (Abdi, 2010). The importance of each parameter was
312 evaluated by its variable importance in projection (VIP) value. Values above 1.0 are considered
313 to have a significant effect on the dependent parameter, whereas values < 0.7-0.8 are not of

statistical significance (Eriksson and Umetrics, 2008). The standardised coefficients of variance X (standardized coefficients calculated after mean-centering and scaling the data to unit variance (Eriksson et al., 2013)) indicate the relative positive/negative effect of their corresponding parameter on the first-order rate constant of drug partition to fat (response value). High absolute values of standardised coefficients for variance X denote a big positive or negative effect on response Y. Outliers in the PLS model were evaluated on the basis of DMoDY (distance to model; residuals of Y), which express distance from each point to the PLS model with respect to the responses with high values.

# 3. Results and Discussion

## 3.1. Effect of fat percentage on drug rate of partition

The rate of drug partition to fat, was highly influenced by the percentage of fat present in the medium, as studied with partition setup I (dialysis membrane). A two-fold increase was observed in the rate constants of partition of nifedipine (drug with the highest fat affinity of the initial five compounds tested) when the fat content was increased from 5 to 25% w/v in the total medium (lipid and milk) volume (Figure 1a). The rate constant of nifedipine partition was doubled when fat concentration increased from 5 (0.05 $h^{-1}$ rate constant) to 25% w/v (0.1 $h^{-1}$ rate constant) when partition data were fitted to a first-order equation, which can be attributed to the larger available area for diffusion when higher fat volumes are used, as described by Fick's first law of diffusion (Erdélyi and Beke, 2003). For the three higher percentages used though (15, 20 and 25% w/v), the rate constants lied approximately between 0.08 and 0.1 $h^{-1}$ (Figures 1a, b). This signified a reduced effect of the fat percentage in partition rate constant for high fat medium content values. By increasing the amount of fat present in the receptor compartment, the percentage of total drug partitioned to fat in a period of 24 h also increased from 58% to 88% for 5% w/v and 25%w/v fat respectively.

### 3.2. Effect of hydrodynamics on drug rate of partition

The evaluation of the effect of agitation in the drug partition setup II, using danazol as model drug for the study is presented in Figure 2. Using increased agitation rates (200 and 250 rpm), almost 100% of the drug was diffused in the lipid layer during the first 30 min. Such high agitation would be difficult to use with model drugs which partition to fat faster or equally fast as danazol, as it would possibly provide inadequate discrimination among them; It can be seen that the profiles in the two high agitation rates were similar (Figure 2) and that the maximum portioned percentage is reached in the first 15 min. When the dissolution apparatus paddle was rotated at 150 rpm, a significantly slower partition profile was acquired with approximately 60% of the drug partitioned from the $FeSSGF_{sk}$ to the lipid compartment in the first hour, and 96% of the drug partitioned in the lipid layer in 8 h (Figure 2). Drug diffusion to the lipid layer is regulated by two static diffusion layers developed in the two sides of the oil-"aqueous" interface with drug diffused through them from the aqueous to the lipid part (Mudie et al., 2012). Assuming that the width of the two layers remains constant though time, the parameters affecting the partition behaviour are: liquid viscosity, vessel dimensions, type of agitator and agitation speed, with the latter being the only parameter changing in the current study, justifying the differences observed in the rate constants of drug partition (Mudie et al., 2012).

### 3.3. Effect of experimental setup on drug rate of partition

The 25% w/v fat percentage was the percentage selected for the evaluation of the different proposed setups for the discrimination of partition rate constants to fat between drugs, even though the % w/v fat in the FDA high-fat standard breakfast (Klein et al., 2004) or in fed gastric media used *in vitro* such as milk, FeSSGF (Fed State Simulated Gastric Fluid) (Jantratid et al., 2008) and Ensure® Plus (Franek et al., 2014) is lower (≈ 1.8-14% w/v). The high fat percentage provided the highest partition rate constant compared to the other fat concentrations

studied (Figure 1b), making the discrimination among the various drugs easier, as observed from the results of the pilot study in setup I.

The five model drugs which were evaluated in the pilot study, using all three setups (Figure 3), all provided significantly different partition rate constants to fat ($p < 0.05$, two-way ANOVA) (Figure 4). In all setups, nifedipine showed the highest affinity for the lipid phase, while the whole amount of propafenone hydrochloride practically remained in the "aqueous" part throughout the duration of the study, possibly because of the latter's low distribution constant ($\log D = 0$) in the working pH.

Using setup I, the whole process was extremely slow for the four of the five model compounds of the study, with the exception of nifedipine, with the amount of drug partitioned to the fat being < 20% in the first 8 h (Figure 3a). A plateau of the percentage partitioned could not be reached even after 48 h for all the model compounds, while phase separation of the milk-based medium was observed after 2 days. The decreased rate constant of the drugs' partition process can be attributed to the increased viscosity of the receptor (fat), which slowed down drug diffusion (Xu et al., 2012). The slow reaction rate constants (especially for compounds other than nifedipine) may be considered a disadvantage for the current setup. Moreover, using the above setup, no significant differences were observed ($p < 0.05$) among the rate constants of drug partition for the five model drugs used (Figure 4).

Setup II resulted in the highest partition rate constants for the five drugs initially studied (Figure 3b). Discrimination among the partition rate constants of the model compounds to fat was observed (Figure 4). The drugs' partition rate constants to fat ranged between 0.39 $h^{-1}$ (for ketoconazole) and 13.58 $h^{-1}$ (for nifedipine). The volumes used were similar to the fed gastric volume *in vivo* (Koziolek et al., 2014; Kwiatek et al., 2009), but the hydrodynamics of this set up are different from the hydrodynamics observed in the fed stomach, as portrayed by the

386 differences in Reynold's number between the fed stomach and the vessels in dissolution
387 studies. USP 2 dissolution apparatus, at speeds between 50 and 100 rpm, results in Re numbers
388 between 5000 and 10000 (Mudie et al., 2010), while the equivalent values of the fed stomach
389 *in vivo* are between 0.01 and 30 (Abrahamsson et al., 2005).

390 Since the stationary level of the fluid is multiple times higher in the USP 2 dissolution
391 apparatus, and is the factor with the biggest effect, (as the medium is the same in both cases),
392 it would be reasonable to assume that $R_e$ values in the setup III model are probably closer to
393 the values resulting from the hydrodynamics developed in the fed stomach *in vivo*. The partition
394 rate constants to the fat for the drugs studied in setup III were: 0.22 (± 0.06), 0.20 (± 0.07),
395 0.33 (± 0.07) and 1.64 (± 0.04) $h^{-1}$ for ketoconazole, atorvastatin calcium, danazol and
396 nifedipine respectively, while propafenone's transfer to fat was insignificant (Figure 3c).
397 Adequate descrimination between the drugs' partition profiles, lower medium volume and drug
398 consumption and reproducible results were obtained with this experimental set up (Figure 4).
399 Therefore, it was selected for the investigation of partition to fat for the rest of the drugs. Setup
400 III partition data were successfully fitted to the first-order equation model, with $R^2$ values of
401 0.90-1.00 and residuals randomly scattered (Figure 3c, Table 4). The highest partition rate
402 constants were observed for nifedipine (1.64 $h^{-1}$) and ibuprofen (1.17 $h^{-1}$), followed by
403 indomethacin (0.70 $h^{-1}$) and griseofulvin (0.63 $h^{-1}$) (Table 4). It can be observed that the four
404 drugs partitioned to the lipid part the fastest are of intermediate lipophilicity (log $D_{pH\ 5}$ = 2.20-
405 3.62). Our hypothesis is that the increased partition rate constants of drugs of moderate
406 lipophilicity is attributed to a combination of adequate drug affinity to fat and also high drug
407 amount available in soluble form in the aqueous donor compartment. The above hypothesis is
408 based on the principles which govern the incorporation of lipophilic drugs in previously formed
409 liposomes, where despite drug increased drug lipophilicity, its rate of incorporation is
410 controlled by the amount of drug available in the aqueous donor phase, with drug dissolution

411 in it being the rate limiting step, often leading to very slow rates if not adequate (Cannon et al.,
412 2008). The decreased drug solubility in the medium may explain the absence of drug partition
413 to fat for MK-C4, the most lipophilic drug in the current study (Figure 3c, Table 4). The rate
414 constants of partition of the other four drugs of high lipophilicity (drug represented with red
415 markers in partition profiles, Figure 3c) ranged from approximately 0.2 to 0.4 $h^{-1}$. The absence
416 of partition (rate constant ≅ 0 $h^{-1}$) for propafenone hydrochloride and furosemide (Figure 3,
417 Table 4) was attributed to decreased drug lipophilicity (log D < 1.5) in the working pH. The
418 percentage of the total drug partitioned to the fat in the duration of the study (8 h) was > 78%
419 for 9 of the 15 model drugs (Figure 3c, Table 4). The lower $F_{8h}$ percentages were observed for
420 drugs of low lipophilicity (propafenone hydrochloride, furosemide, phenytoin, indoprofen) and
421 can be attributed to their low affinity for fat. Drug transfer process between these two
422 immiscible layers is governed by three steps; firstly, its diffusion towards the interface, its de-
423 and re-solvation at the interface and, lastly, a new diffusion step from the interface to the lipid
424 layer (Grassi et al., 2002). Therefore, the insignificant partition of the drug with the highest log
425 D, MK-C4, could possibly be explained by the drug's limited ability to dissolve in the
426 "aqueous"/organic interface.

427 **3.4. Effect of drug physicochemical properties on drug rate of partition**

428 The relationship between drug partition rate constants and drug lipophilicity (log $D_{pH}$
429 $_5$) follows a bell-shaped distribution around a maximum of log D = 2-4 (Figure 5). Several
430 moderately lipophilic drugs (nifedipine, ibuprofen) of the study partitioned to fat faster than
431 others of higher lipophilicity (felodipine, itraconazole) indicating that lipophilicity is not the
432 sole parameter affecting the process.

433 Partition data (rate constants) showed that molecules of molecular weight higher than
434 500 g/mol partition to the lipid layer of the gastric medium at a slow rate relatively to the other

model drugs, despite their high lipophilicity (e.g. atorvastatin calcium, ketoconazole, itraconazole, MK-C4) (Figure 5). Ionisation is also a parameter affecting drug partition into the lipid phase; for ionisable compounds, their un-ionised form is more easily partitioned to the lipid membranes (Fan and de Lannoy, 2014). It can be assumed that having the model drug in its un-ionised from in the aqueous donor ($FeSSGF_{sk}$) would facilitate its partition to the lipid layer. Out of the 15 model drugs of the study, the ones being ionised at a percentage higher than 95% in the working pH (according to their pKa values, Table 2) demonstrated the lowest partition rate constants, regardless of their lipophilicity (Tables 2, 4, Figure 5).

The impact of aqueous solubility at the working pH, lipophilicity and MW and *in vivo* food effect on drug partition behaviour was evaluated using partial least squares regression analysis. The initial PLS analysis for the rate constant of partition (1 principal component) which included all model drugs of the study, gave a model of moderate predictive power ($Q^2$ = 0.33) and fit ($R^2$ = 0.43) to the experimental values. Of the 15 drugs, nifedipine behaved as an outlier, with partition rate constants significantly higher than the model predicted (DMoDY values for nifedipine were 2.2-2.4 times higher than the critical value given by the software for the specific PLS model). Nifedipine's affinity to fat can be seen in the lipid percentage-dependent solubility of the drug in fed gastric media *in vitro*, as derived from its values in fed gastric media of 3.5, 1.75 and 0.875% w/v fat, (approximately 12x, 7x and 4x solubility for early, middle and late FeSSGF compared to aqueous buffers of the same pH and buffer capacity) (Andreas et al., 2016). Fast partition to fat could be one of the reasons of nifedipine being a drug which exhibits positive food effect when administered with high fat meals (Schug et al., 2002) with more drug being solubilised by the lipid content of the gastric environment after meal administration. Reconstructing the model without including nifedipine gave a model defined by 2 principal components of $Q^2$ and $R^2$ values of 0.55 and 0.66 respectively, with acceptable predictive power.

Molecular weight (VIP = 1.0) was defined as negative predictor for drug partition to fat (negative standardised coefficient) (Figure 6). Molecular weight > 500 is considered a limiting negative factor for drug permeation to lipid membranes through passive diffusion (Banks, 2009; Lipinski et al., 2001). As observed by the negative standardised coefficient of the log $D_{pH\ 5}$*MW interaction (VIP = 1.3), the effect of lipophilicity is different for drugs of different molecular weights. Looking at the drug partition rate constants (Table 4), it can be demonstrated that even extremely lipophilic drugs which do not have a reasonably low molecular weight, cross the lipid-aqueous interface barrier at a low rate (Figure 5). Positive log $D_{pH\ 5}$*aq $sol_{pH\ 5}$ interaction (VIP = 1.1) indicates that the effect of drug lipophilicity on the rate constant of partition to fat differs according to drug aqueous solubility. Positive food effect is generally associated with drug lipophilicity though lipid emulsification of lipophilic drugs in the stomach (Porter et al., 2008) and increase in drug luminal solubility (Porter and Charman, 2001). The model build failed to demonstrate any correlation between positive food effect and drug-fat interaction, as the statistically non-significant negative coefficient indicates (Figure 6). On the contrary, the model demonstrated a negative correlation (Figure 6) between partition rate constants to fat with negative food effect *in vivo* (VIP = 1.2). Drugs of which pharmacokinetic behaviour does not change as a result of meal administration (no food effect), appear to partition to fat significantly faster than the rest (positive standardised coefficient, VIP = 1.7), which is another indication that partition to fat alone cannot be used as a sole predictor for changes in drug pharmacokinetic parameters after administration of high-fat meals.

### 3.5. Effect of emulsification conditions on drug rate of partition

When 1% w/v of SLS was added to the drug donor, in order to simulate an extreme version of the emulsification of the fat taking place in the fed gastric environment, enhancement of the drug partition rate constants to fat was observed for 9/15 drugs following first-order kinetics both in presence and absence of surfactant. The increase in the rate constant of the

485 partition process ranged from 11.4% (indoprofen) to 335.8% (felodipine), while a slight
486 decrease compared to the partition rate constant in the absence of SLS was reported for three
487 of the drugs (4.7, 10.0 and 29.5% decrease in partition rate constants for ibuprofen, griseofulvin
488 and itraconazole respectively) (Figure 7a, Table 4). The presence of SLS in the drug donor
489 compartment had a statistically significant effect on the drug partition rate constants, compared
490 to the profiles in the absence of surfactant ($p < 0.05$). The difference in partition rate constants
491 to fat is a result of two conflicting phenomena; i. higher drug affinity for the donor in the
492 presence of surfactant and ii. bigger available receptor surface area for partition compared to
493 the control experiment. The addition of SLS under constant stirring breaks the fat into smaller
494 droplets which increases their surface area (Gunstone, 2007). In the fed stomach, where fat
495 emulsification takes place, as a result of the agitation conditions and the presence of lipid
496 digestion products, the diameter of fat droplets is significantly reduced with the emulsion
497 surface area demonstrating a three-fold increase (Schulze, 2006), which justifies the increase
498 in partition rate constants in this study, after the addition of SLS. The presence of surfactants
499 (emulsifiers, proteins or lipolysis products) in the lipid-water interface can reduce the surface
500 tension increasing the diffusion kinetics by increasing the drug interfacial permeability,
501 compared to the large surface tension of the non-emulsified lipid-water interface, where
502 partition phenomena are slow (Rezhdo et al., 2016).

503 The partition process is drug dependent with significant difference observed among
504 different drugs ($p < 0.05$). Higher rate constants of partition to fat were observed for moderately
505 lipophilic drugs ($\log D_{pH\,5}$ values 2-4) and lower rate constants for drugs of extreme low or
506 high lipophilicity (Figure 8a). A bell-shaped curve is observed for the correlation of partition
507 rate constants with $\log D_{pH\,5}$ (Figure 8a).

508 The PLS analysis for the rate constants of partition (1 principal component) which
509 included all model drugs of the study except for nifedipine, gave a model of good predictive

power ($Q^2$ = 0.41) and fit ($R^2$ = 0.51) to the experimental values. Absence of food effect (VIP = 1.9) and the interaction of log $D_{pH\ 5}$ with negative food effect (VIP = 1.1) were defined as positive predictors for drug partition to fat (positive standardised coefficients) (Figure 9). Faster partition rate constants to fat both in presence and absence of the surfactant, in drugs of similar bioavailability in fasted and fed conditions, is another indication that drug rate partition to fat is not directly associated with *in vivo* positive food effect. On the contrary, a negative correlation between positive *in vivo* food effect can be observed from the negative standardised coefficient (VIP = 1.4) in the model (Figure 9). In the fed stomach, during gastric emptying, aqueous content is transferred to the duodenum significantly faster than fat, which is held by the angular notch (Schulze, 2006). Therefore, if the drug is adequately soluble in the watery portion of the gastric content, aided by the natural surfactants present (a role played by the surfactant in the developed *in vitro* partition setup), slower partition to the lipid layer would mean increased drug quantity available for transfer to the intestinal environment. A slower partition to fat therefore, provided that the drug is adequately soluble and does not precipitate in the aqueous part of the stomach, may be associated with positive food effect. Negative effect of drug aqueous solubility (VIP = 1.1) is attributed to the high affinity of highly soluble drugs for the "aqueous" compartment. The impact of drug lipophilicity on the rate constants of partition to fat differs according to drug molecular weight and drug aqueous solubility, as indicated by the significance of the log $D_{pH\ 5}$ andMW (VIP = 1.1) and log $D_{pH\ 5}$ andaq sol $_{pH\ 5}$ (VIP = 1.1) interactions.

In the presence of the lipase, the differences in partition behaviour among drugs of different lipophilicity were subtler (Figure 8b) than in the presence of SLS. Rate constants of partition ranged from 0.207 $h^{-1}$ (atorvastatin calcium) to 1.354 $h^{-1}$ (nifedipine) (Table 4). Although partition rate constants for all drugs were equally high or slightly higher than in the absence of the enzyme (Table 4, Figure 3c, 7b), the differences were not statistically significant, which

implied that the partition rate constants to fat were not affected by the presence of the lypolytic enzyme. MK-C1 and nifedipine demonstrated slightly slower rate constants than in the absence of lipase by 1.11 and 1.21 times respectively. Similarly, to the other two conditions (absence of surfactant/enzyme and presence of SLS), drugs of intermediate lipophilicity (blue markers in Figure 7b) partition to fat faster and to a higher percentage than extremely lipophilic (red markers) and hydrophilic (white markers) drugs. Except for the three most hydrophilic drugs (propafenone hydrochloride, furosemide, indoprofen) and the extremely lipophilic MK-C4, a percentage higher than 80% of all drugs partitioned to the fat layer in a period of 8 hours except for itraconazole (Figure 7b, Table 4). Interestingly, in the presence of lipase, itraconazole started diffusing to fat only after 3 hours and only 32.4% of the initial drug concentration partitioned to fat in 8 hours.

The bell-shaped distribution around log D values of 2-4, (Figure 8b) indicates that in the presence of lipase, drug partition behaviour is not only governed by the drug lipophilicity, but it can be also controlled by other physicochemical parameters, an effect confirmed by the multivariate analysis of data. Looking at the partition rate constants against MW and un-ionised drug fraction plots (Figure 8b), the rate of partition seems to be affected by MW and drug ionisation to a smaller extent.

The PLS analysis for the rate constants of partition (1 principal component) which included all model drugs of the study except for nifedipine, gave a model of good predictive power ($Q^2 = 0.55$) and fit ($R^2 = 0.65$) to the experimental values. The main variables with a negative effect on drug rate constants of partition were drug MW (VIP = 1.2), $\log D_{pH\,5}$*MW interaction (VIP = 1.8) and drug aqueous solubility (VIP = 1.1) (Figure 10). The negative effect of the former is attributed to the negative correlation of molecular weight on drug diffusion coefficient (Valencia and González, 2011). The effect of $\log D_{pH\,5}$ differs with MW, as the negative $\log D_{pH\,5}$*MW interaction indicates. Drug aqueous solubility is negatively correlated

with drug partition rate constant due to higher affinity for water-soluble compounds for the donor compartment. The positive standardised coefficient of $\log D_{pH\,5}$*aq $sol_{pH\,5}$ (VIP = 1.3) indicates that a possible positive correlation of drug partition rate constant with its lipophilicity is dependent on drug aqueous solubility. This positive coefficient could be an indication that lipophilicity is a parameter favourably affecting the partition process, provided that the drug is adequately soluble in the donor compartment. The absence of *in vivo* food effect was a significant variable in the model (positive standardized coefficient, VIP = 1.3), indicating that the drug rate partition constant to fat is not directly associated with the impact of meal presence on drug bioavailability.

## 4. Conclusions

Drug interaction with fat has been closely related to possible *in vivo* food effect after drug administration with meals of high lipid content. The current study strove to depict the drug partition process to the lipid phase of the fed stomach content by developing an *in vitro* discriminating method, able to assess the differences in rate constants of drug partition to fat using model drugs of a wide range of lipophilicity, ionisation and food effect. The *in vitro* setup developed provided discrimination of drug partition rate constants. The study revealed that increasing the percentage of fat and the lipid and aqueous compartment volumes while reducing the agitation conditions resulted in significantly enhanced the partition rate constants. Drugs of moderate lipophilicity partitioned to fat faster compared to low or high lipophilic drugs. The study revealed the importance of drug physicochemical properties, as the rate constants of drug partitioned to fat depended also on drug MW and drug aqueous solubility. The complex role of drug partition to fat was depicted by the significance of several interaction terms using multivariate data analysis. Faster drug partitioning to fat can be anticipated when surfactants are included in the setup due to fat emulsification. The current investigation revealed a correlation between drugs demonstrating negative food effect and slow partition to fat.

However, it is important to highlight that food effect on bioavailability depends on a number of complex mechanisms and cannot be predicted solely based on the elucidation of lipid-drug interactions in the fed stomach. Moreover, conclusions on drug partition rate constants must not only be based on API properties but the effect of drug formulation must also be considered. Further studies, investigating also the drug "release" from the lipid part of the gastric fed content to the intestinal environment combining the partition principles of developed setup with simulated intestinal media could potentially shed light towards better understanding of food effect mechanisms.

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855 **Tables**

856 **Table 1:** Physicochemical properties and working concentrations of model drugs; "+" indicates positive food effect, "-"negative food effect and

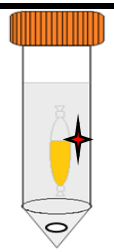
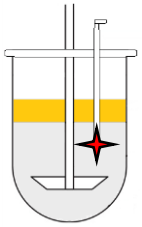
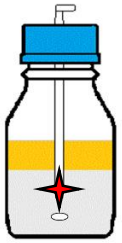
857 "n.e." no food effect.

| Drug | Food effect | log P | log D $_{pH\ 5}$[a] | pKa (Law et al., 2014) | Un-ionised fraction $_{pH\ =\ 5}$ (%) | Molecular Weight (g/mol) | Working concentration (µg/mL) |
|---|---|---|---|---|---|---|---|
| **Furosemide** | - (Beermann and Midskov, 1986) | 2.29 (Granero et al., 2010) | 1.46 | 4.25 (acidic) | 15 | 331 | 80 |
| **Griseofulvin** | + (Aoyagi et al., 1982) | 2.50 (Fagerberg et al., 2015) | 2.50 | - | 100 | 353 | 8.6 (Wishart et al., 2006) |
| **Phenytoin** | + (Hamaguchi et al., 1993) | 2.47 (Fleisher et al., 1990) | 2.47 | 9.47 (acidic) | 100 | 253 | 27 (Mithani et al., 1996) |
| **Ibuprofen** | n.e (Pargal et al., 1996) | 3.97 (Potthast et al., 2005) | 3.62 | 4.85 (acidic) | 45 | 206 | 84 |
| **Indoprofen** | n.e (Tamassia et al., 1977) | 2.50 (Fagerberg et al., 2015) | 1.21 | 3.74 (acidic) | 5 | 281 | 128 (Wishart et al., 2006) |
| **Nifedipine** | + (Schug et al., 2002) | 2.20 (Gajendran et al., 2015) | 2.20 | 13.00 (basic) | 100 | 346 | 10 (Nokhodchi et al., 2008) |
| **Propafenone hydrochloride** | + (Axelson et al., 1987) | 3.39 (Cramer et al., 2007) | 0 | 9.63 (basic) | 0 | 341 | 150[b] |
| **Ketoconazole** | - (Männistö et al., 1982) | 4.40 (Diakidou et al., 2009b) | 2.64 | 6.75 (basic) | 2 | 531 | 2.7[b] |
| **MK-C1**[c] | n.e | 4.00 | 4.00 | 6.50 (acidic) | 100 | 777 | 3[c] |
| **Danazol** | + (Charman et al., 1993) | 4.20 (Fagerberg et al., 2010) | 4.20 | - | 100 | 337 | 1 (Dressman and Reppas, 2000; Fagerberg et al., 2010) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Atorvastatin calcium** | n.e (Radulovic et al., 1995) | 4.82 (Rageh et al., 2017) | 4.06 | 4.33 (acidic) | 18 | 559 | 2.6[b] |
| **Indomethacin** | n.e (Aoyagi et al., 1990) | 3.50 (Fagerberg et al., 2015) | 2.27 | 3.80 (acidic) | 6 | 358 | 15 (Fagerberg et al., 2010) |
| **Felodipine** | n.e (Weitschies et al., 2005) | 4.80 (Fagerberg et al., 2015) | 4.80 | - | 100 | 384 | 1.1 (Fagerberg et al., 2010) |
| **Itraconazole** | + (Barone et al., 1993) | 6.20 (Maincent et al., 2017) | 5.60 | 3.70 (basic) | 23 | 706 | 3.7[c] |
| **MK-C4[c]** | + | 8.81 | 8.81 | - | 100 | 638 | 3.2[d] |

[a] Calculated based on equations 1 and 2
[b] Working drug concentrations based on calculated drug aqueous solubility values (Advanced Chemistry Development (ACD/Labs) Software v.11.02, Sci-finder)
[c] Physicochemical properties and food effect data provided by Merck
[d] Solubility study (24 h) performed in $FeSSGF_{sk}$

862 **Table 2**: Experimental conditions of drug partition to fat setups.

| Setup | I | II | III |
|---|---|---|---|
| Anhydrous milk fat<br>$FeSSGF_{sk}$<br>Sampling point<br>Magnetic stirring bar |  |  |  |
| **$FeSSGF_{sk}$ volume (donor) (mL)** | 30 | 500 | 30 |
| **Anhydrous milk fat nominal quantity (g)** | 10.3 | 172 | 10.3 |
| **Anhydrous milk fat nominal concentration (% w/v)** | 5-25 | 25 | 25 |
| **Sampling compartment** | Centrifuge tube (50 mL) | Paddle apparatus vessel | Glass bottle (100 mL) |
| **Sampling point** | Middle of $FeSSGF_{sk}$ layer | | |
| **Sample volume (mL)** | 0.5 | 5 | 0.5 |
| **Agitation speed (rpm)** | 300 | 150 | 300 |
| **Membrane length (cm)** | 8 cm | - | - |
| **Membrane type** | Cellulose membrane avg. flat width 25 mm (1.0 in.), MWCO 14000 | - | - |
| **Sampling time points (h)** | 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 24, 48* | 0.17, 0.33, 0.5, 1, 1.5, 2, 3, 4, 6, 8 | 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 |
| **Temperature (°C)** | 37 | | |

863 *24 h sampling time for nifedipine, 48h sampling time for all other drugs

**Table 3:** HPLC methods (or modification of published methods) used for the quantification of the model compounds

| Drug | Column | Mobile phase | Flow rate (ml/min) | Temperature (° C) | Inj. Vol. (µl) | λ (nm) |
|---|---|---|---|---|---|---|
| **MK-C4*** | Phenomenex Onyx monolithic $C_{18}$, 300Å, 250 x 4.6 mm, 5 µm | ACN:$H_2O$ 70:30 | 3.5 | 40 | 100 | 220 |
| **Itraconazole (Vertzoni et al., 2006a)** | Thermo Hypersil BDS $C_{18}$, 300Å, 250 x 4.6 mm, 5 µm | MeOH:$H_2O$ 80:20 | 1 | 35 | 100 | 260 |
| **Felodipine (Diakidou et al., 2009a)** | Waters XBridge Shield RP18, 130Å, 150 x 4.6, 3.5 µm | ACN:$H_2O$ 70:30 | 1 | 25 | 100 | 238 |
| **Indomethacin (Maharaj et al., 2016)** | Waters XBridge Shield RP18, 130Å, 150 x 4.6, 3.5 µm | MeOH:Phosphoric acid 1.67% v/v | 1 | 23 | 50 | 270 |
| **Atorvastatin calcium (Elshanawane et al., 2014)** | Thermo Hypersil BDS $C_{18}$, 300Å, 250 x 4.6 mm, 5 µm | ACN:Phosphate buffer 0.025 M (pH 6) 40:60 | 1.5 | 30 | 100 | 246 |
| **Danazol (Zhang et al., 2008)** | Thermo Hypersil BDS $C_{18}$, 300Å, 250 x 4.6 mm, 5 µm | MeOH:$H_2O$ 75:25 | 1 | 25 | 100 | 285 |
| **MK-C1*** | Waters Symmetry Shield $C_{18}$, 100Å, 50 x 4.6 mm, 5 µm | ACN:Phosphate buffer 0.025 M (pH=2.5) Gradient (0-2 min 65:35/ 2-2.01 min 90:10/ 2.01-3 min 90:10/ 3-3.01 min 65:35 | 3 | 40 | 100 | 214 |
| **Ketoconazole (Diakidou et al., 2009b)** | Thermo Hypersil BDS $C_{18}$, 300Å, 250 x 4.6 mm, 5 µm | MeOH:$H_2O$:DEA 75:25:0.1 | 1 | 25 | 100 | 260 |
| **Propafenone hydrochloride (Borijihan et al., 2014)** | Agilent Eclipse XDB $C_{18}$, 120Å, 250 x 4.6 mm, 5 µm | MeOH:ACN: TEA:$H_2O$ 50:7.5:0.1:q.s 100 (pH 2.9) | 0.8 | 25 | 20 | 248 |
| **Nifedipine (Vertzoni et al., 2006b)** | Thermo Hypersil BDS $C_{18}$, 300Å, 250 x 4.6 mm, 5 µm | MeOH:$H_2O$ 60:40 | 1 | 20 | 50 | 238 |
| **Indoprofen (Locatelli et al., 2014)** | Waters XBridge Shield RP18, 130Å, 150 x 4.6, 3.5 µm | ACN:Formic acid 0.1% v/v 40:60 | 1 | 40 | 50 | 280 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Ibuprofen (Tan et al., 2014)** | Agilent Eclipse XDB $C_{18}$, 120Å, 150 x 4.6 mm, 5 µm | MeOH:Acetic acid 0.2% v/v 60:40 | 1 | 25 | 100 | 233 |
| **Phenytoin (Maharaj et al., 2016)** | Agilent Zorbax SB-$C_{18}$, 150 × 4.6 mm, 3.5 µm | ACN:$H_2O$ 80:20 | 1 | 20 | 10 | 210 |
| **Griseofulvin (Maharaj et al., 2016)** | Waters XBridge Shield RP18, 130Å, 150 x 4.6, 3.5 µm | MeOH:$H_2O$ 65:35 | 0.8 | 20 | 50 | 292 |
| **Furosemide (Sora et al., 2010)** | Thermo Hypersil BDS $C_{18}$, 300Å, 250 x 4.6 mm, 5 µm | MeOH:Formic acid 0.1% v/v 60:40 | 0.8 | 25 | 20 | 233 |

865 *HPLC methods provided by Merck

866 **Table 4** Drug first-order partition rate constants to fat ($k_{part}$) and % drug partitioned to fat at 8h ($F_{8h}$) in the absence and presence of SLS and lipase.

| Drug | No emulsifier | | SLS | | Lipase | |
|---|---|---|---|---|---|---|
| | $k_{part}$ ($h^{-1}$) | $F_{8h}$ (%) | $k_{part}$ ($h^{-1}$) | $F_{8h}$ (%) | $k_{part}$ ($h^{-1}$) | $F_{8h}$ (%) |
| propafenone hydrochloride | 0 (0)* | - | 0 (0) | - | 0 (0)* | - |
| furosemide | 0 (0)* | - | 0 (0) | - | 0 (0)* | - |
| indoprofen | 0.429 (0.049) | 19.4 (7.1) | 0.477 (0.087) | 25.0 (0.6) | N/A | 30.8 (1.9) |
| ketoconazole | 0.223 (0.037) | 80.0 (1.2) | N/A | 18.4 (1.8) | 0.825 (0.002) | 97.2 (0.8) |
| griseofulvin | 0.628 (0.059) | 84.1 (3.6) | 0.565 (0.045) | 41.7 (2.4) | 1.036 (0.058) | 95.5 (3.3) |
| phenytoin | 0.364 (0.039) | 59.7 (3.3) | 0.592 (0.103) | 48.0 (2.4) | 0.617 (0.155) | 90.1 (2.4) |
| ibuprofen | 1.165 (0.073) | 95.8 (0.5) | 1.111 (0.163) | 86.0 (1.0) | 1.228 (0.065) | 95.9 (0.46) |
| danazol | 0.330 (0.043) | 93.5 (0.9) | 1.033 (0.213) | 86.7 (1.8) | 0.673 (0.036) | 100 (0) |
| nifedipine | 1.642 (0.022) | 100 (0) | 3.748 (0.134) | 82.3 (1.2) | 1.354 (0.018) | 100 (0) |
| indomethacin | 0.697 (0.095) | 90.4 (1.7) | 2.344 (0.760) | 75.6 (3.2) | 1.109 (0.240) | 95.4 (2.1) |
| atorvastatin calcium | 0.198 (0.043) | 41.6 (11.5) | 1.122 (0.211) | 23.2 (6.6) | 0.207 (0.087) | 82.1 (4.3) |
| MK-C1 | 0.404 (0.036) | 88.5 (14.1) | 1.212 (0.513) | 90.4 (1.0) | 0.365 (0.044) | 88.5 (1.7) |
| felodipine | 0.390 (0.005) | 88.4 (0.8) | 1.699 (0.406) | 87.9 (2.7) | 0.584 (0.166) | 98.9 (2.0) |
| itraconazole | 0.335(0.020) | 78.6 (2.8) | 0.236 (0.044) | 100 (0) | N/A | 32.4 (2.5) |
| MK-C4 | 0 (0)* | - | 0.703 (0.066) | 93.0 (2.8) | 0 (0)* | - |

867 *partition was insignificant; partition rate constants were considered zero

868 N/A: the first-order model did not fit to the partition data

## Figure captions

**Figure 1: a.** Nifedipine partition profiles to fat using setup I (dialysis membrane) and different fat percentages. Dashed lines denote the fittings to the first-order model. **b.** Bars denote the first-order partition rates of nifedipine partition to fat in setup I.

**Figure 2:** Danazol partition profiles to fat using the setup II under different agitation conditions.

**Figure 3:** Drug partition profiles to fat using **a.** setup I, **b.** setup II and **c.** setup III. The marker colour is representative of drug lipophilicity (log $D_{pH\ 5}$); white colour for the five more hydrophilic drugs, blue colour for the five moderately lipophilic and red for the five most lipophilic. Dashed lines denote the fittings to the first-order model.

**Figure 4**: Calculated first-order rates of drug partition studies. Stars denote statistical differences among setups (two-way ANOVA, Bonferroni post-hoc test, * < 0.05, ** < 0.01, *** < 0.001). Different letters denote statistically significant differences ($p < 0.05$) among partition rates within the same setup.

**Figure 5:** Drug partition rates to fat in the absence of SLS or RN lipase vs log $D_{pH\ 5}$, MW, and % of un-ionised drug in the working pH.

**Figure 6:** Standardised coefficients corresponding to the variables (and their interactions) studied. Green colours denote coefficients of VIP > 1.

**Figure 7:** Drug partition profiles to fat using the setup III in the presence of **a.** SLS or **b.** lipase. The marker colour is representative of drug lipophilicity (log $D_{pH\ 5}$); white colour for the five more hydrophilic drugs, blue colour for the five moderately lipophilic and red for the five most lipophilic. Dashed lines denote the fittings to the first-order model.

**Figure 8:** Drug partition rates to fat **a.** in the presence of SLS or **b.** RN lipase, vs log $D_{pH\ 5}$, MW and % un-ionised drug at pH 5.

**Figure 9:** Standardised coefficients corresponding to the variables (and their interactions) studied. Green colours denote coefficients of VIP > 1.

**Figure 10:** Standardised coefficients corresponding to the variables (and their interactions) studied. Green colours denote coefficients of VIP > 1.

899 Figure 1

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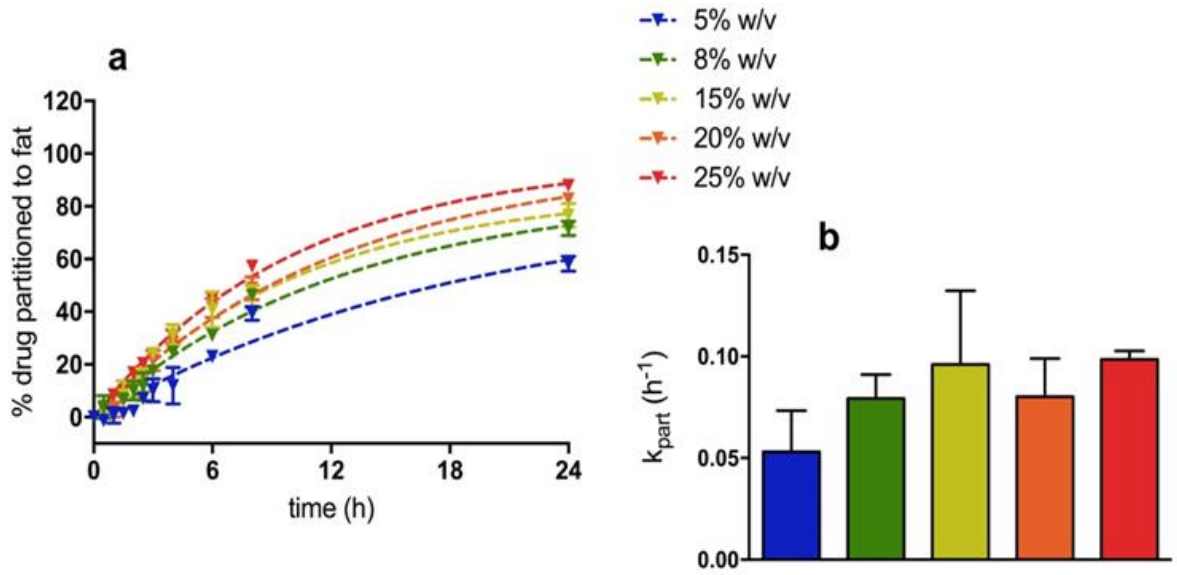


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904 Figure 2

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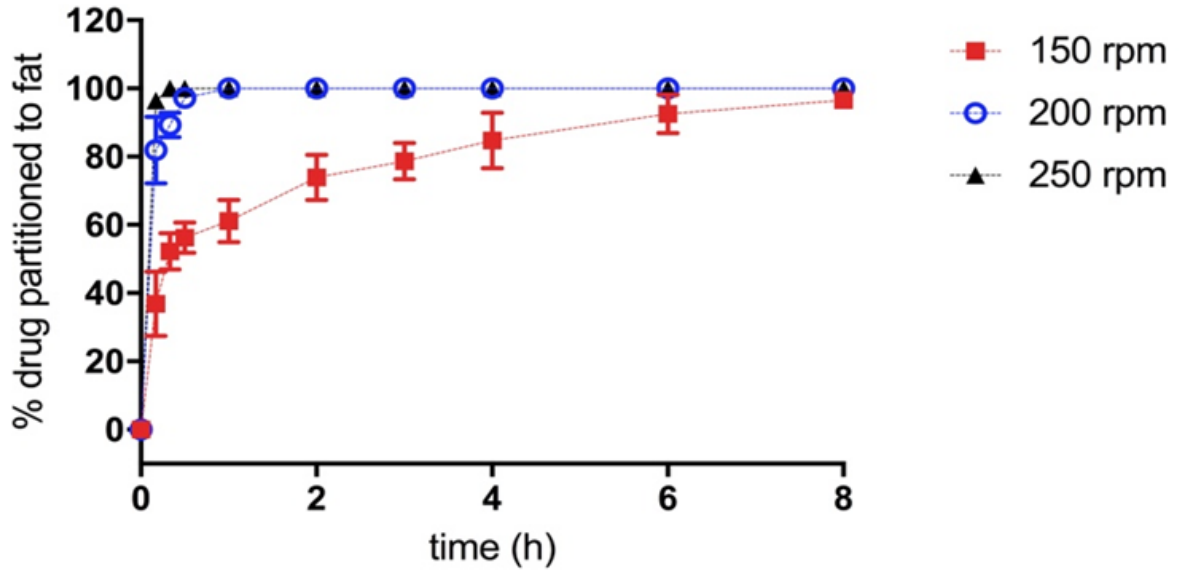


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909 Figure 3

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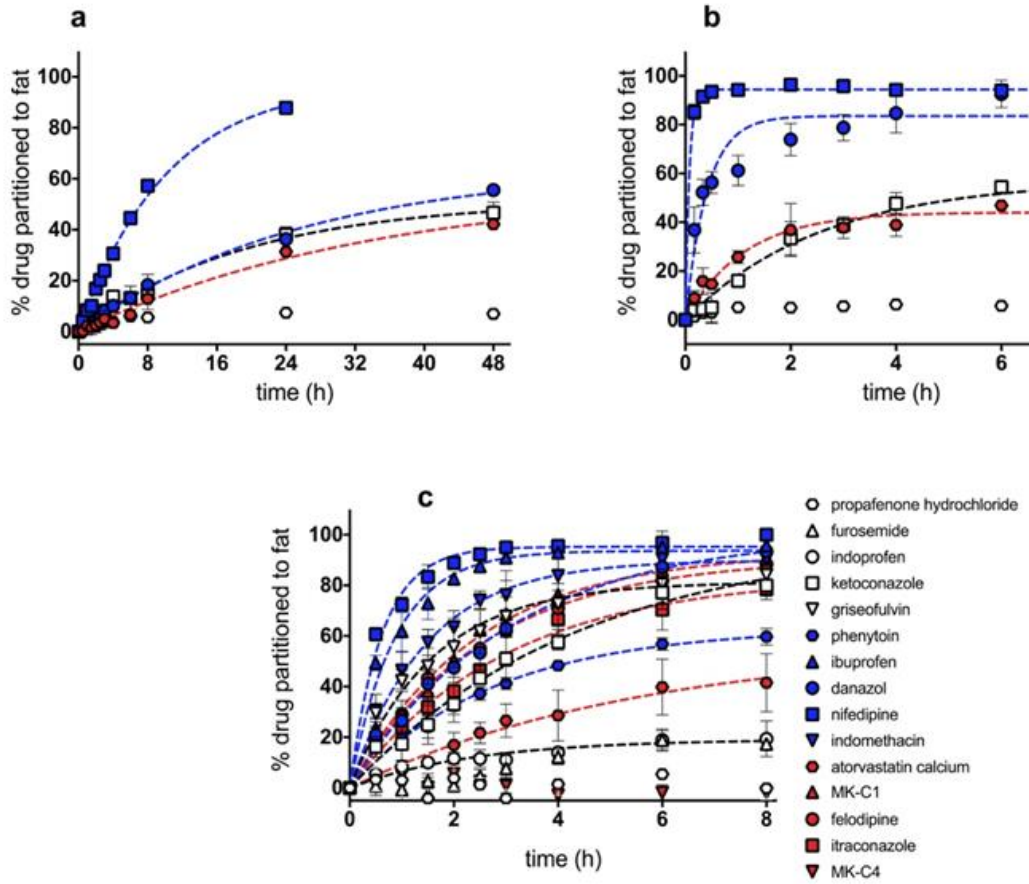


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914 Figure 4

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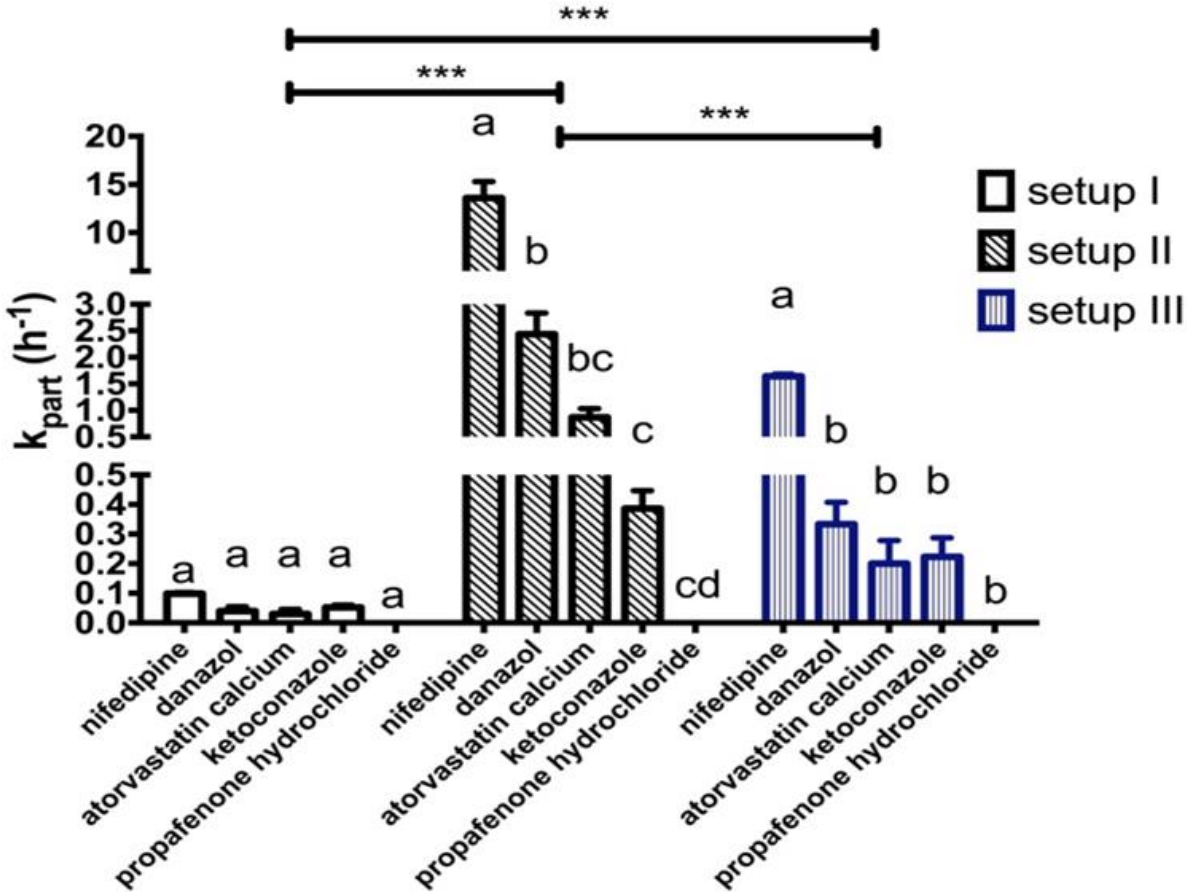


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920 Figure 5

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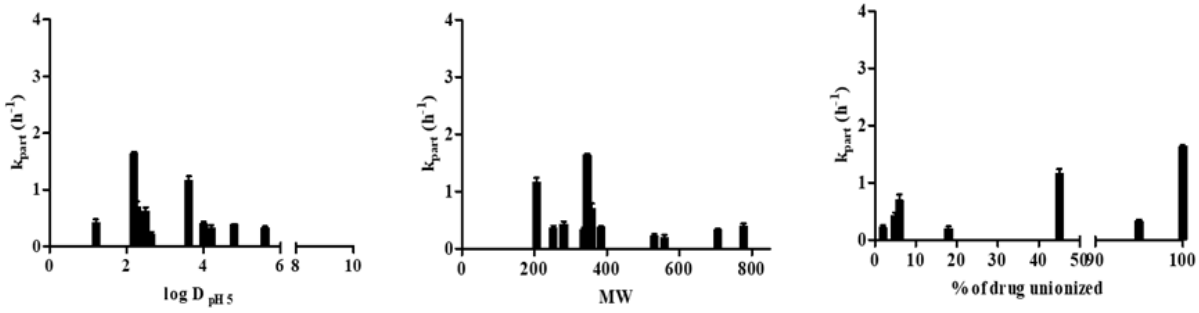


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925 Figure 6

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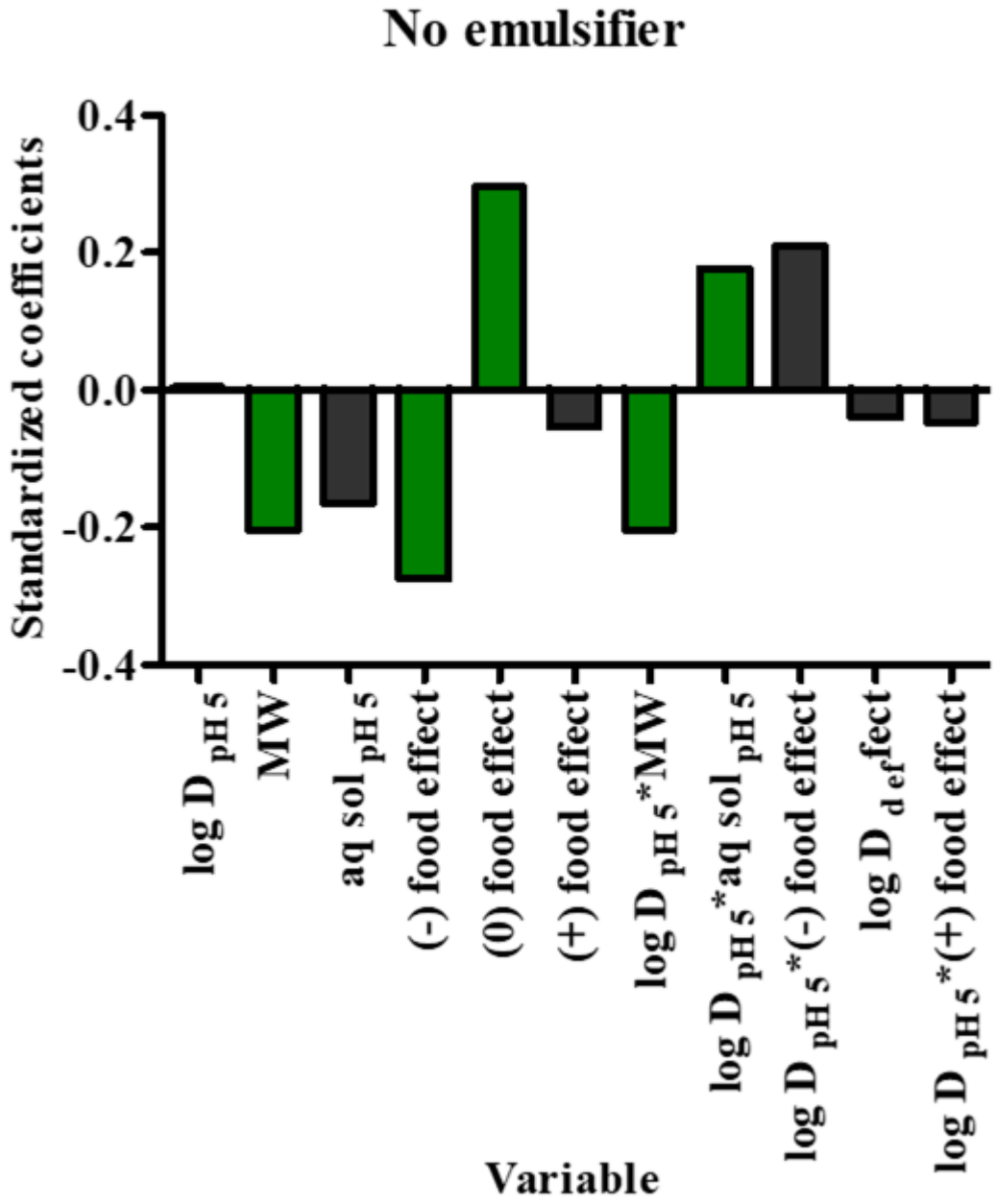


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930 Figure 7

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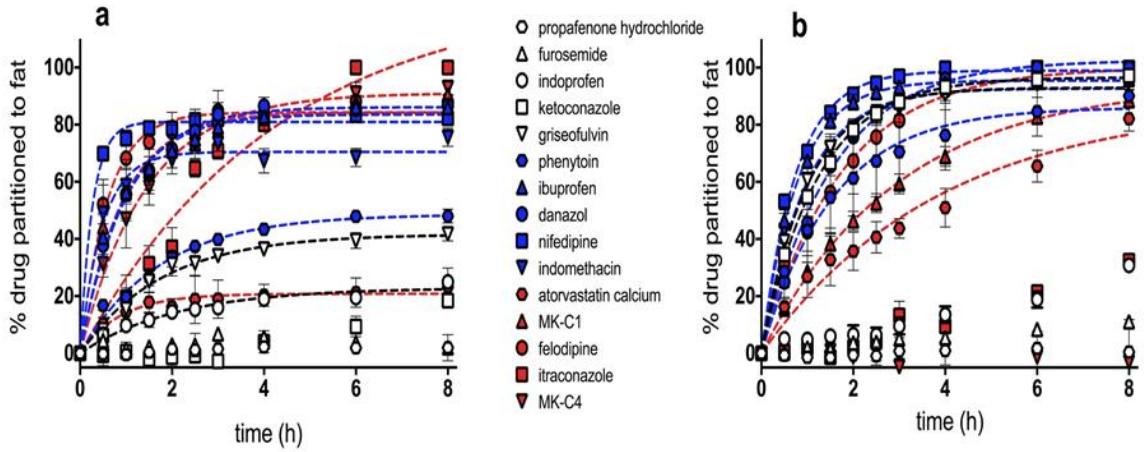


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935 Figure 8

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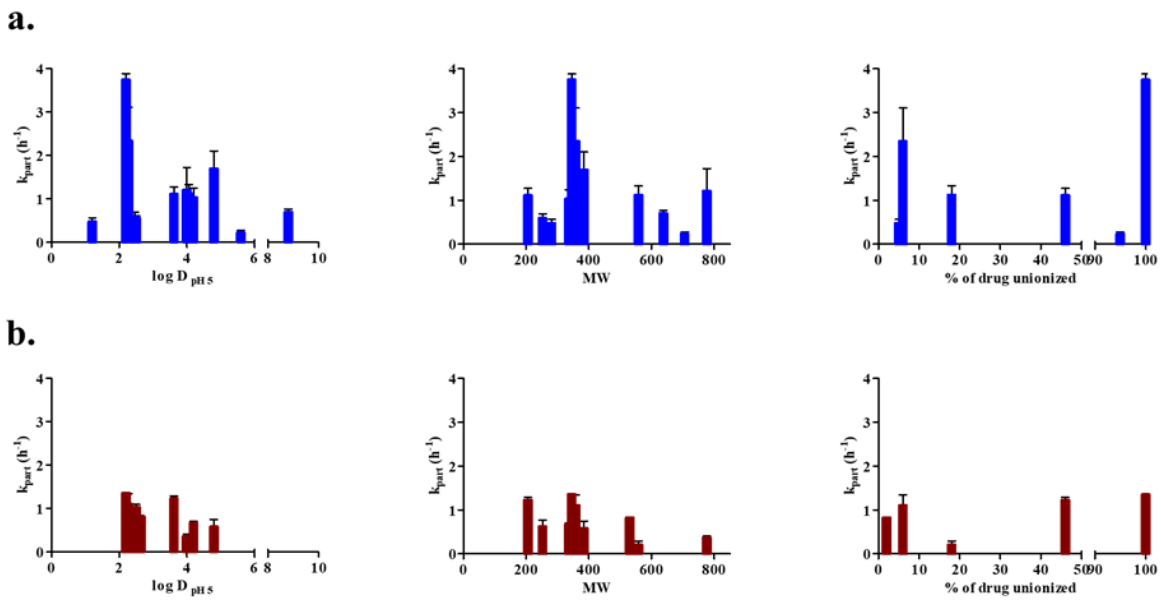


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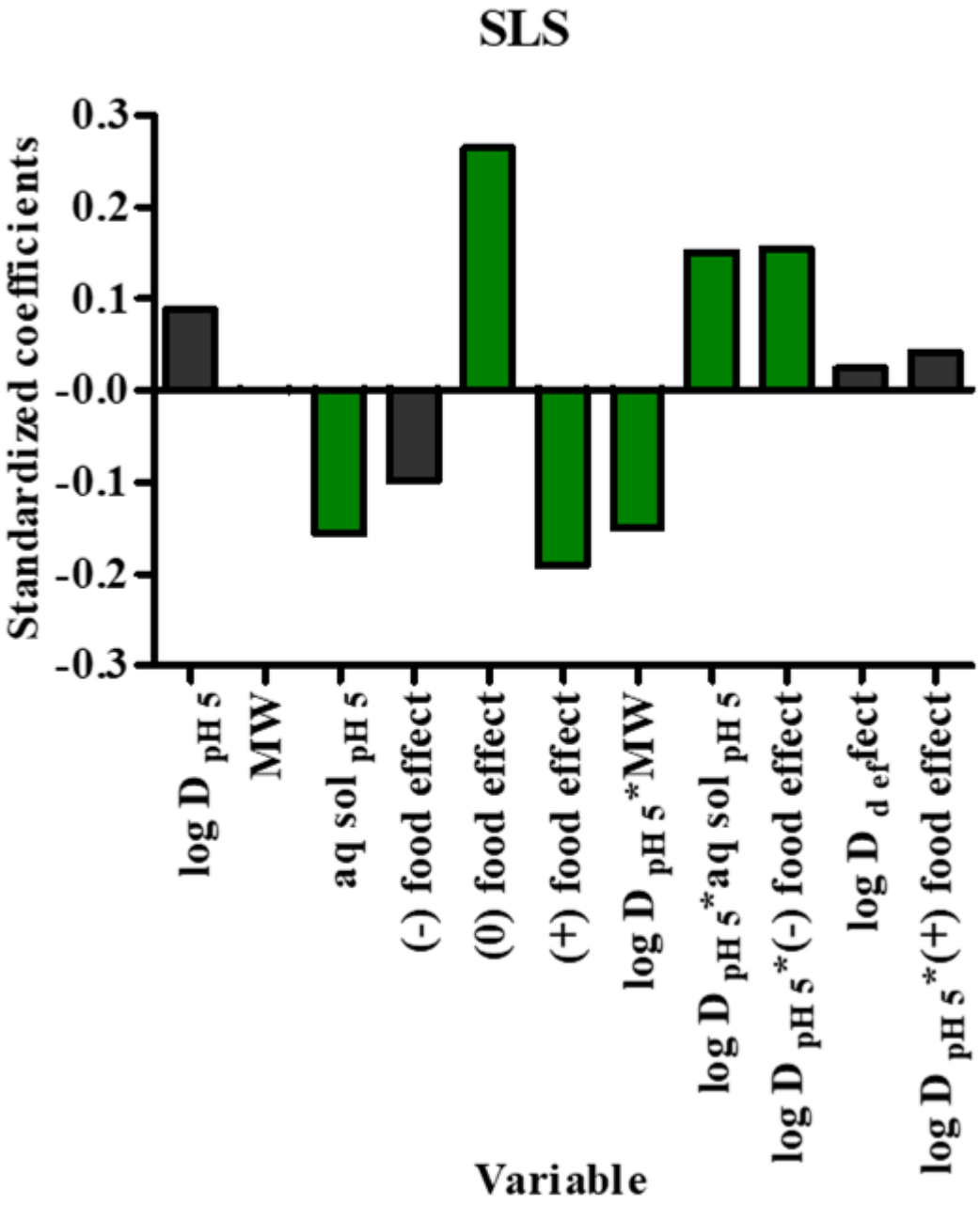
940 Figure 9

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945 Figure 10

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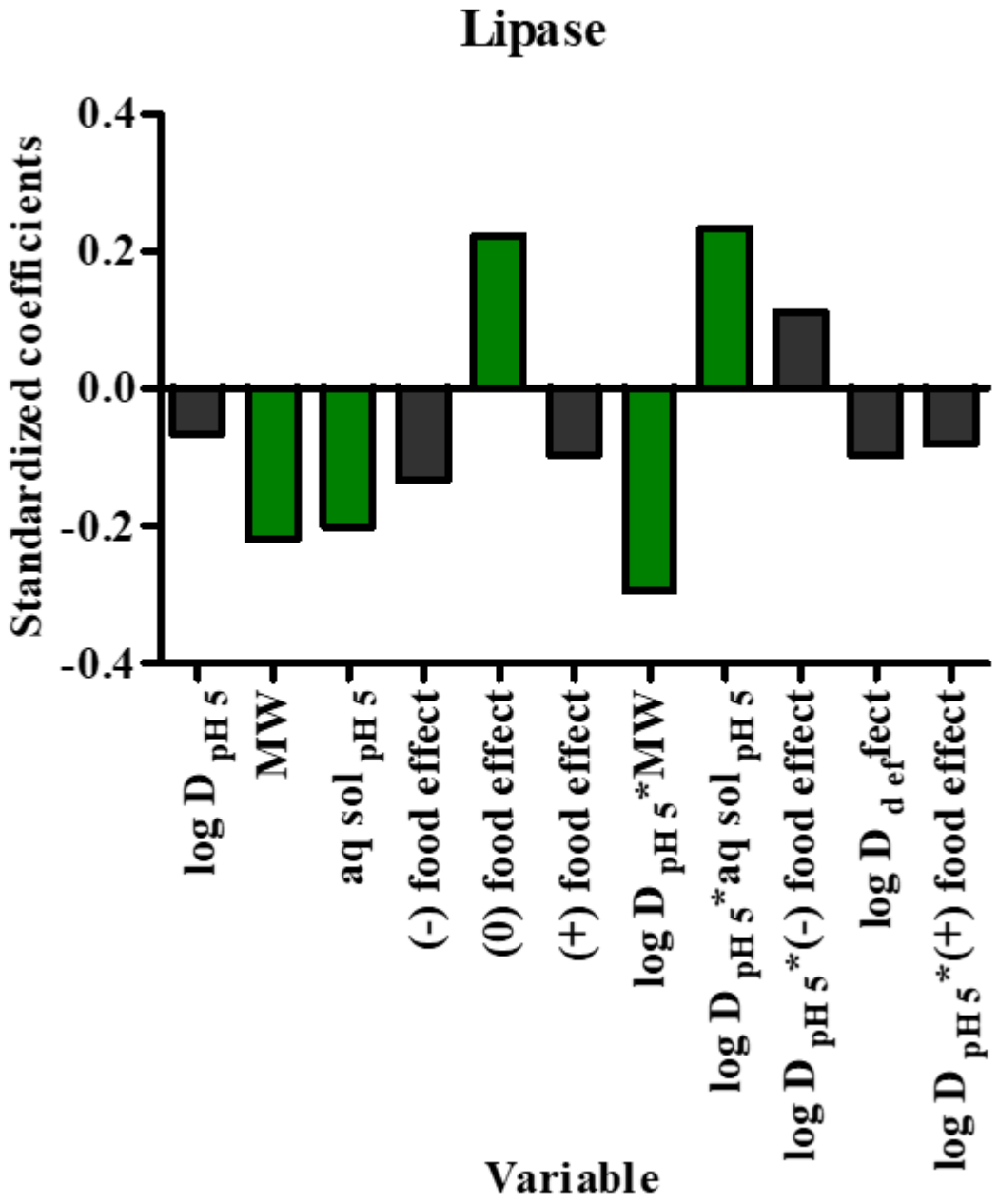


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